

POSTER PRESENTATION

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Understanding cell behavior in cultivation processes - A metabolic approach

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Background

During cultivation cells undergo a tremendous change in their metabolism when shifting from one state to another or when parameters are changed. To understand the changes in intracellular metabolite concentrations and their impact on cell performance we used a systematic approach. By employing the chemostat mode at different steady state conditions we investigated the alterations of the concentrations of key metabolites during cultivations of a human production cell line.

Methods

Chemostat cultivations were performed with the AGE1.hn AAT cell line (Probiogen AG, Berlin, Germany) and TC-42 medium (Teutocell AG, Bielefeld, Germany) in a fully controlled 2 litre benchtop bioreactor (Sartorius, Göttingen, Germany). Different dilution rates of 0.24 d⁻¹, 0.33 d⁻¹, and 0.40 d⁻¹ and pH values of pH 6.9, pH 7.15, and pH 7.3 were performed using the same bioreactor setup. For stopping the cell metabolism an established fast filtration method [1] was used for rapid quenching. Metabolites were extracted from cells using liquid/liquid extraction. Extracts were analyzed by using hydrophilic interaction chromatography (HILIC) and ESI-MS/MS mass spectrometry. Extracellular amino acids and pyruvate were analyzed by pre-column derivatization and RP-HPLC [2], glucose and lactate using a YSI 2700 bioanalyser.

Results

The comparative analysis of the three steady state dilution rates shows the great impact of changing extracel-

lular conditions on the intracellular metabolite pools which may also lead to an altered productivity. For example, as been shown in Figure 1A the specific pyruvate consumption rate, qPyr, as well as the intracellular pyruvate pools decrease with increasing dilution rates, while qGlc and qGln increase at the same time. While some metabolite pools show great differences between different dilution rates others remain more or less constant. A malonate inhibition of the TCA cycle (Figure 1B) appears mainly at low dilution rates, which might be an effect of glucose and / or glutamine limitation at those steady states.

Although qGlc, qPyr as well as qGln decrease with increasing pH values (data not shown), the intracellular TCA pools remain constant due to a catabolism of further amino acids (Table 1). This may have led to a lower waste of ammonia, lactate and glycine at higher pH values.

The analysis of the intracellular nucleotide pools show that while the concentrations of almost all nucleotides dropped with increasing dilution rates, they were more or less stable at changing pH values (data not shown).

Conclusions

Although more data have to be raised to get a comprehensive insight into cell metabolism it could be shown that chemostat cultures performed at steady state conditions are a valuable tool for investigating cell behaviour on an intracellular basis. A much better data stability can be obtained than in batch or fed-batch cultures.

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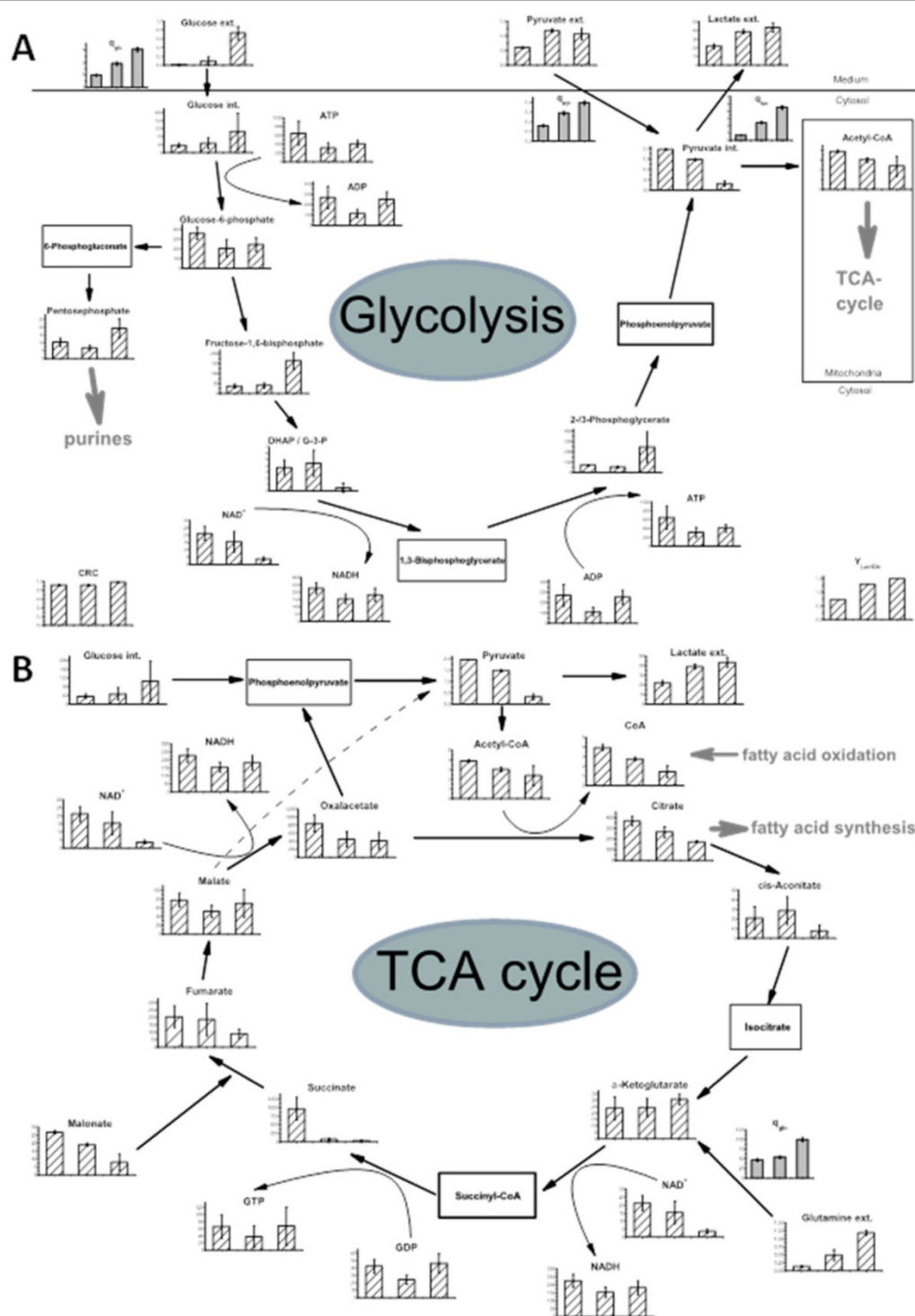


Figure 1 Metabolite pool sizes in Glycolysis (A) and TCA (B) at different dilution rates. The metabolism at the three different dilution rates 0,24 d⁻¹ (left), 0,33 d⁻¹ (middle), 0,4 d⁻¹ (right) is shown. Specific rates are illustrated with filled bars and given in nmol cell⁻¹ d⁻¹. Stripped bars illustrate pool sizes which are given in mM (extracellular) and μM (intracellular), respectively.

Table 1 Correlation of specific rates q_{xxx} with the adjusted pH values during steady state

	pH 6,9	pH 7,15	pH 7,3
Q_{NH3}	430 ± 27	243 ± 9	207 ± 19
Q_{Lac}	4751 ± 298	3766 ± 143	3548 ± 325
Q_{Glc}	- 3660 ± 230	- 3302 ± 126	- 3301 ± 302
Q_{Pyr}	- 155 ± 10	- 121 ± 5	-84 ± 8
Q_{Gln}	- 527 ± 33	- 488 ± 19	- 484 ± 44
Q_{Asp}	- 63 ± 4	- 123 ± 5	-153 ± 14
Q_{Glu}	66 ± 4	29 ± 1	- 16 ± 2
Q_{Asn}	- 17 ± 1	- 42 ± 2	-45 ± 4
Q_{Ser}	-91 ± 6	-198 ± 8	- 191 ± 17
Q_{His}	- 13 ± 1	- 23 ± 1	-5 ± 1
Q_{Gly}	32 ± 2	9 ± 0	7 ± 1
Q_{Thr}	- 26 ± 2	61 ± 2	67 ± 6
Q_{Arg}	- 39 ± 2	- 97 ± 4	- 109 ± 10
Q_{Ala}	101 ± 6	48 ± 2	99 ± 9
Q_{Tyr}	- 10 ± 1	-29 ± 1	29 ± 2
Q_{Met}	-20 ± 1	-39 ± 2	- 40 ± 4
Q_{Val}	-37 ± 2	-79 ± 3	- 88 ± 8
Q_{Trp}	- 5 ± 0	- 8 ± 0	- 9 ± 1
Q_{Phe}	- 10 ± 1	-36 ± 1	-36 ± 3
Q_{Ile}	- 35 ± 2	- 68 ± 3	- 72 ± 7
Q_{Leu}	- 63 ± 4	-111 ± 4	-122 ± 11
Q_{Lys}	- 21 ± 1	- 89 ± 3	- 100 ± 9

The specific rates are given in $\mu\text{mol cell}^{-1} \text{d}^{-1}$. (Negative values indicate consumed metabolites.)

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